REMARKS/ARGUMENTS

Substitute Specification

Appendix A contains a clean copy of the specification. Appendix B contains a marked-up copy of the specification in accordance with 37 C.F.R. 1.125(b). The original specification was condensed by deleting text which was substantially duplicative and reorganizing the text into a more logical order. The marked-up copy of the specification indicates changes made to the original specification, including the cumulative amendments to have been filed for the instant application.

Applicants attest that the information in the substitute specification is supported by the originally filed specification, and that the substitute specification introduces no new matter. Entry is respectfully requested.

Formal Drawings

Applicants also request entry of the set of replacement drawing sheets enclosed in Appendix C. The drawings have been edited to remove redundant figures and renumber the figures in consecutive order. The set of replacement drawing sheets contain no new matter.

Sequence Listing

Applicants attach a paper and CRF of the sequence listing. The sequences is the sequence listing are the same as those disclosed in the application. The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy enclosed herewith. This amendment contains no new matter.

Response to Claims Rejections

In response to the Examiner allegations that certain claims are open to antibodies of arbitrarily high affinities, applicants have amended the independent claims to recite that the affinity constant of the claimed humanized is within about 4-fold of the affinity of the donor antibody. Support is provided at e.g., p. 5, line 34. Claim 116 has been amended to recite that

the humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside the CDRs that replace the corresponding amino acids in the acceptor immunoglobulin framework, consistent with claim 119 and the disclosure of the specification at e.g., paragraph bridging pp. 30-31. Other amendments are for purposes of clarity and are discussed below. No amendment should be construed as an acquiescence in any ground of rejection. Applicants respond to the office action using the paragraph numbering of the office action.

3. A substitute specification is attached and a mark-up showing changes made. The original specification was condensed by deleting text which was substantially duplicative and reorganizing the text into a more logical order. The marked-up copy of the specification indicates changes made to the original specification, except that the section entitled "CROSS-REFERENCE TO RELATED APPLICATIONS" has been further updated in the substitute specification. The cover page of the substitute specification has been amended to reflect a request for a change of inventorship being submitted herewith. Applicants attest that the information in the substitute specification is supported fully in the originally filed specification, and that the substitute specification introduces no new matter.

Applicants also request entry of the attached set of replacement drawing sheets. The drawings have been edited to remove redundant figures and renumber the figures in consecutive order. The set of replacement drawing sheets contain no new matter.

4. A sequence listing is attached. The substitute specification includes the appropriate sequence identifiers.

5. The examiner objects to the term "the amino acid" as lacking antecedent basis in claims 108, 110 and 119. These claims have been amended for improved clarity. The reference to "said position" followed by a Markush group in claim 133 is alleged to be unclear as to whether it refers to any of the three categories of positions or just category III. In response, the description of the Markush group has been moved to an earlier point of the claim to indicate with improved clarity that the group is applicable to each of the three categories.

6. Claims 109, 111, 117, 120, 128 and 131 stand rejected under 35 USC 112, first paragraph as failing to comply with the written description requirement. The rejection is based on recitation in the claims of an antibody having an affinity constant of at least 10^8 M^{-1}, and the Examiner's belief that a higher affinity constant means a lower affinity. In response, it is believed that the rejection stems from some confusion between a dissociation constant and an affinity constant. A dissociation constant (Kd) is expressed in units of molarity, M. A representative dissociation constant of an antibody is 10^{-9} M. The smaller the dissociation constant, the higher the affinity. On the other hand, an association or affinity constant (Ka) is the reciprocal of a dissociation constant and is expressed in units of inverse molarity, M^{-1}. A representative affinity constant of an antibody is 10^9 M^{-1}. An affinity constant of an antibody is sometimes referred to simply as the "affinity" of the antibody. The larger an affinity constant, the higher the affinity of the antibody. The above claims refer to an affinity constant, not a dissociation constant. Thus, the recitation of antibodies having an affinity constant greater than at least 10^8 M^{-1} is consistent with the disclosure of the application. It is noted that analogous language has been used in the claims of predecessor patents (see, e.g., claim 1 of US 5,585,089). Accordingly, withdrawal of the rejection is respectfully requested.

7. Claims 108-135, 137-172, 174-191, 193-201 and 203-20 stand rejected under 35 USC 112, first paragraph on the basis that although the specification is enabling for humanized immunoglobulins having affinities within 2- to 4-fold the affinity of the parent antibody, it does not provide enablement for higher affinities relative to the parent immunoglobulin. The Examiner alleges that the specification does not teach how to improve the affinity of exemplified immunoglobulins over the parent, and in fact, some of the exemplified humanized immunoglobulins have affinities two-fold less than the parent.

In response, the independent claims have been amended to recite an upper limit of affinity of within about 4-fold of that of the donor immunoglobulin. This amendment is made simply to speed allowance and should not be construed as acquiescence in the rejection.

It is noted that a similar rejection was made in predecessor case 08/484,537 and was subsequently withdrawn in view of applicants' arguments (see paper 37 at p. 6). The

application issued as US 6,180,370 and many of the claims (see, e.g., claims 10, 11 and 13) have no restriction on affinity. Applicants reiterate the arguments that prevailed in the '537 application to clarify the basis for their position in the present record.

Although the specification does disclose some exemplary humanized antibodies in which the affinities of the humanized antibodies are slightly lower that those of the corresponding donor antibodies, these examples should not be considered limiting--particularly as the subject specification does disclose additional examples with significantly increased affinity over the donor. Subsequent publications by the inventors and others provide additional evidence that skilled artisans were able to routinely practice the present invention and obtain humanized antibodies exhibiting increased affinity in comparison to the donor antibody.

Humanized immunoglobulins made in accordance with the present invention have been shown experimentally to have affinities much stronger than their donor immunoglobulin. For example, Co et al. (1992) *J. Immunology* 148:1149-1154 (CN on IDS) and Caron et al. (1992) *Cancer Research* 52:6761-6767 (CE on IDS), describe humanized antibody affinities from about 3 to 8 fold stronger than the donor M195 antibody. Specifically, Co. et al. reported "about a 3-fold higher" affinity (see, bottom right hand column of page 1152 continued on page 1153). Similarly, Caron et al. reported the humanized antibodies "showed up to an 8.6- and 4-fold higher binding avidity" (see, Abstract, line 12). Both papers name original co-inventor Queen as an author, and describe humanized antibodies either precisely disclosed in the subject specification (e.g., humanized IgG_1, M195 described at specification pages 148-153) or otherwise made using the same humanized heavy chain variable domain with a different heavy chain constant domain (e.g., humanized IgG_3, M195; see, Co et al., page 1150, "Construction of expression vectors").

As another example, WO 96/05229 (BX on IDS), reports humanizing the mouse antibody M1129 using the strategy and principles of the present application. For example, heavy and light chain variable region frameworks were selected based on a high degree of sequence identity to the corresponding frameworks in the donor antibody (see pp. 9-10). Donor residues were then substituted at four positions in the heavy chain variable region framework and one

position in light chain variable region framework (see specification at p. 20, second paragraph). The application indicates that the Kd's for the mouse (M1129) and humanized antibodies (H1129) are 11.4 nM for M1129 and 1.4 nM respectively (see p. 29). As the affinity (association) constant K_a is always the reciprocal of the dissociation constant K_d, this means that the affinity of M1129 is $8.8 \times 10^7 M^{-1}$, whereas the affinity of H1129 is $7.1 \times 10^8 M^{-1}$, about 8-fold greater. The applicants of WO 96/05229 did not do anything beyond the express teachings (particularly the criteria) of the present application to increase the affinity of the humanized antibody, i.e., they did not perform in vitro mutagenesis, affinity maturation, or the like. On the contrary, these applicants simply followed the criteria and other teachings of the present specification in a straightforward manner, which according to them yielded a humanized antibody with much greater affinity than the donor mouse antibody. In conclusion, the methods of the present application sometimes generate humanized antibodies with affinity slightly lower than the donor antibodies, more often with affinity essentially the same as the donor antibodies, sometimes with affinities higher but no greater than 4-fold, and sometimes with affinities even higher than that.

Although the above evidence shows the methods disclosed in the specification can result in humanized antibodies having greater affinities than the donor antibody, the Examiner appears to assume that there exists a class of humanized antibodies having still greater affinity that could be made by some other unspecified method, but not by the methods disclosed in the specification. Applicants disagree that such a class of humanized antibodies is reasonably expected to exist. Rather, it is Applicants' position that the extent of affinities attainable in humanized antibodies using the methods disclosed in the present specification is substantially coextensive with affinities that inherently reside in humanized antibodies encompassed by the claims at issue. Thus, the enablement provided by the specification is commensurate with the scope of the claims.

In the prior case, 08/484,537, in which essentially the issue of an upper limit for affinities was previously considered, the Examiner cited a reference, Groves et al., Hybridoma 6,

71 (1987) (DH on IDS) reporting the isolation of a sheep monoclonal antibody having an affinity of the order of 10^{11} M^{-1}, presumably, on the rationale that if one can make a sheep antibody with an affinity of 10^{11} M^{-1}, then it should be possible to make any humanized antibody having the same affinity. This rationale is mistaken. The high affinity of the sheep antibody reported by Groves presumably resides in the residues that principally effect binding, namely, the CDR region and a few variable region framework amino acids that interact with the CDRs. Although it should be possible to humanize Grove's particular antibody and achieve an affinity of 10^{11} M^{-1}, this does not mean that donor antibodies in general can be humanized with such affinities. A humanized antibody incorporates CDR regions and key framework residues from the particular donor antibody being humanized, and thereby precludes incorporation of the same residues from a different donor, such as Grove's sheep antibody.

For these reasons, it is reasonably expected that such inherent features of a donor as may limit the affinities of humanized antibodies produced by the present methods, apply equally to other methods, and that the enablement provided by the specification is thus commensurate with claim scope. In these circumstances, it is unnecessary and unreasonable to expect applicants to identify a precise point of demarcation between feasible and infeasible affinities and expressly recite this in the claims so as to exclude infeasible affinities. In general, the claims serve to define what an invention is, rather than what an invention is not. For any claimed invention, one can identify an infinite number of theoretical embodiments that in all likelihood could never be made in practice. For example in the present case, one could theoretically imagine humanized antibodies that are resistant to autoclaving or which simultaneously cure two unrelated diseases, but it would almost certainly be impossible to make any such antibody. To expect applicants to identify all such theoretically imaginable but practically impossible embodiments at the time of filing, and expressly exclude them from the claims would be an impossible and unreasonable burden, and is not the law.

It is perhaps for this reason that numerous US patents have issued which claim compositions and methods of humanizing or otherwise engineering antibodies while maintaining their affinity, without any explicit exclusion of antibodies exceeding an upper limit of affinity

(see, e.g., US 5,766,886, US 5,225,539, US 5,639,641 and US 5,859,205, US 6,407,213, and most recently US 6,639,055 issued Oct. 28, 2003). In each case, it is clear that some upper limit must exist. Nevertheless, it would have been an unreasonable and unnecessary burden to expect the patentees to have defined precisely what the limit was, and such was not required.

8. Claims 133, 138, 142, 148, 150, 158-162, 164, 166-168, 170, 175, 177 and 199 stand rejected under 35 USC 112 first paragraph for alleged failure to comply with the written description requirement. The Examiner states that some of the framework substitutions appear to have been made at positions not contemplated by the specification, and that certain framework positions such as H66 and H103 appear to be within CDR regions. The Examiner acknowledges applicants' remarks concerning the difference between sequential and Kabat numbering but states that applicants have not described which positions are originally described by the sequential method and which by the Kabat method.

In response, it is noted that Kabat numbering is used for the substitutions listed at p. 33 of the specification (as is stated at p. 33, line 28), and in the Table at p. 127 (as stated under the Table) and corresponding Figs. 35A-D. Use of Kabat numbering in Figs. 35A-D is apparent from the assignment of certain amino acid positions with numbers and letters (e.g., 27a, 27b, 27c and 27d in Fig. 35B) rather than sequential numbers. On the other hand, in other references to substitutions (all occurring in the Examples and the corresponding Table 1 at p. 54), the specification uses the sequential numbering system. Such is apparent from the sequential numbering of positions in the figures referenced in the Examples (e.g., Figs. 1, 2, 3, 4, 5, 6, 15, 16, 30, 44, 50, and 56). Sequential numbering can be recognized readily by the assignment of successive numbers to every successive amino acid in an immunoglobulin chain in these figures (in contrast to the use of both numbers and letters for certain positions in Kabat numbering, as is used in Figs. 35A-D). For example, the specification states that the substitutions listed in Table 1 at p. 54 are numbered the same way as in Figs. 2-6 (see p. 53, lines 8-10). Because Figures 2-6 can clearly be seen to employ the sequential numbering system, the positions referred to in Table 1 must do so as well.

That one of ordinary skill in the art has no difficulty appropriately converting the sequentially numbered positions of the present application to Kabat numbering is evident from the prosecution history of an application to similar subject matter by a third party. Carter et al., USSN 08/146,206 (now US 6,407,213), is an application with a later effective priority date also directed to humanized immunoglobulins incorporating certain substitutions of donor residues at variable region framework positions. In an attempt to show that their substitutions were different from those disclosed by a predecessor patent of the present application, US 5,530,101 (which constituted prior art to their '206 application), Carter et al. provided several tables converting the sequential numbering of the present application to Kabat numbering. A copy of the relevant part of the Carter et al. prosecution history including these tables **is attached** (paper 32 of Carter et al. at pp. 6-10).

It can be seen that the position numbers of all of the substitutions listed in the present claims (except for H71) occur in columns titled "Kabat numbering" in the attached tables. These Kabat position numbers correspond precisely to the substitutions disclosed by the present application appearing in the adjacent column titled "sequential numbering." Position H71 (by Kabat numbering) is not listed in the tables, but is disclosed in the present specification as being a position for substitution (p. 33, line 27). Therefore, the present specification does disclose all substitutions referred to in the claims. Furthermore, positions H66 and H103 (which the present claims refer to by Kabat numbering) do not fall within CDR regions when the CDR regions are likewise defined by Kabat numbering. So defined, the heavy chain CDRs occupy positions 31-35, 50-65 and 95-102 of the heavy chain (see specification at p. 127, lines 34-35).

For these reasons, it is submitted that the specification demonstrates applicants' possession of all substitution positions listed in the claims.

9-12. Claims 108-132 stand rejected under the judicially created doctrine of obviousness-type double patenting over US 6,180,370, US 5,530,101, US 5,593,762 and/or US 5,585,089. Simply to expedite prosecution and because a patent issuing from the present application will in any event have a shorter term that any of the cited patents, applicants are

prepared to file a terminal disclaimer on indication of otherwise allowable subject matter. Applicants do not acquiesce in the merits of the rejections.

13. Claims 116, 119, 122 and 123 stand rejected under 35 USC 102(a) as anticipated by Riechmann. The Examiner interprets the term "consensus framework from many human antibodies" broadly as including frameworks having only a single substitution exchanging a rare amino acid for a more typical amino acid or formed from a combination of at least two antibodies. Riechmann is said to teach grafting hypervariable regions from a rat antibody into both human NEW and REI frameworks. The Examiner states that the REI framework appears to be a consensus framework because the legend refers to it as being "based on" REI and therefore in the Examiner's view is presumably a combination of two or more antibodies. The Examiner also states that Riechmann makes Ser 27 and Ser 30 substitutions. This rejection is respectfully traversed.

All of the claims at issue specify that the heavy chain variable region framework is a consensus sequence, optionally with donor amino acids replacing corresponding amino acids from the consensus sequence at certain position. In independent claim 119, the requirement for a heavy chain acceptor sequence is explicit. In independent claim 116, the requirement is implicit in that the claim refers to light and heavy chain variable region frameworks collectively as the acceptor variable region framework, and specifies that this framework is a consensus sequence. As will be shown, Riechmann does not disclose a heavy chain variable region framework acceptor that is a consensus sequence, as this term is properly construed.

In the absence of explicit contrary intent, claim terms take on their customary meaning as given by persons experienced in the field of the invention *Hoechst Celanese Corp. v. BP Chems. Ltd.*, 78 F.3d 1575, 1578 (Fed. Cir. 1996). The customary meaning of the term "consensus sequence" was discussed in prosecution of predecessor cases (see, e.g., USSN 08/474,040 paper 15 at p. 14) and applicants reiterate these remarks here. The term "consensus sequence" was, and is, understood in the art to denote the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related sequences. *See, e.g.*, Winnaker, *From Genes to Clones* (Verlagsgesellschaft, Weinheim, Germany, 1987), at p. 240

(discussing diversity between promoter sequences and explaining that a "consensus sequence comprises the most frequently occurring nucleotides"). In a family of proteins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence.

At the priority date of the invention, many such consensus sequences, including some immunoglobulin consensus sequences had been published. For example, Kabat et al., *Sequences of Proteins of Immunological Interest* (4th ed., 1987), which was the primary reference for matters related to immunoglobulin sequences at the priority date of application, and which was incorporated in the application by reference at p. 27, lines 4-5 of the specification, presents a list of the most frequent amino acids at each position in the variable region for each subgroup of light and heavy chains. (The classification of immunoglobulins into subgroups of light and heavy chain variable regions is explained at p. 31, lines 29-34 of the subject patent application.) The framework amino acids in each such list provided by Kabat thus constitute a consensus framework of many human antibodies. For example, a consensus framework of the many human antibodies with a light chain in subgroup 1 is listed on p. 45 of Kabat et al., op. cit.

Yet further support for applicants' position regarding the skilled person's understanding of "consensus sequence" is provided by Carter et al. US 6,407,213, which states at col. 11, lines 26 – 30: "The terms 'consensus sequence' and 'consensus antibody' as used herein refers to an amino acid sequence which comprises <u>the most frequently occurring amino acid residues at each location</u> in all immunoglobulins of any particular subclass or subunit structure." (emphasis added). Notably, this definition, which matches that of the applicants, is made in the same context of humanized immunoglobulins as that of the subject application. In contrast, the Examiner has not cited any use of the Examiner's expansive concept of consensus sequence by those of skill in the art.

The cited Riechmann reference does not disclose or suggest using an acceptor immunoglobulin heavy chain variable region framework having a consensus sequence, as this term was used and understood in the art at the priority date of the claimed invention (or at

present). The heavy chain variable region framework used by Riechmann is from the particular human NEW antibody and not from a consensus sequence. That Riechmann made two substitutions of this acceptor sequence at positions 27 and 30, one of which was apparently to introduce a more commonly occurring amino acid, is certainly insufficient to make Riechmann's acceptor variable region framework a consensus sequence, as many other amino acids in the NEW heavy chain framework are *not* the most frequently occurring amino acids at their positions among human immunoglobulins.

Riechmann's reference to a light chain variable region framework "based on" the REI antibody also does not anticipate the claims. It is not at all apparent from the words themselves that a light chain variable region acceptor "based on" REI means a consensus sequence. "Based on" REI could include REI itself or other variants thereof besides a consensus sequence. However, even if it is assumed *arguendo* that the acceptor immunoglobulin *light chain* variable region framework used by Riechmann did have a consensus sequence, such would not constitute an anticipation of the present claims, which as noted require use of an acceptor immunoglobulin *heavy chain* variable region framework having a consensus sequence.

Claim 119 and claim 116 (as amended) are distinguished from Riechmann for the additional reason that the claims require that amino acids from the donor immunoglobulin replace corresponding amino acids from the acceptor immunoglobulin framework having a consensus sequence. If the Phe's introduced at positions H27 and H30 by Riechmann are considered to be part of the acceptor framework, as appears to be the Examiner's position, then they cannot also constitute substitutions of that framework by donor amino acids.

14. Claims 116-132 stand rejected under 35 USC 102(e) as anticipated by Huston, US 5,476,786. The Examiner alleges that Huston teaches humanized immunoglobulins comprising consensus sequences in view of his reference to humanized immunoglobulins that comprise frameworks that are homologous with a portion of the frameworks of a human immunoglobulin (citing to col. 2, line 60-col. 3, line 40). Huston is also alleged to cite the general concept of replacing an amino acid in a framework region of an acceptor immunoglobulin from a donor immunoglobulin for the purpose of increasing binding specificity

of the immunoglobulin (citing to col. 13, lines 45-57, col. 7, lines 40-54) and to provide a working example although not in the context of a humanized antibody (citing to col. 13, lines 16-38). This rejection is respectfully traversed.

Huston does not anticipate any of claims 116-132 because Huston does not disclose use of a consensus sequence. "Anticipation is established only when a single prior art reference discloses, expressly or under principles of inherency, each and every element of a claimed invention," RCA Corp v. Applied Digital Data Sys. Inc. 2212 USPQ 385, 388 (Fed. Cir. 1984). "Inherency ... *may not be established by probabilities or possibilities.*" *Mehl/Biophile v. Milgraum,* 52 USPQ2d 1303, 1305 (Fed. Cir. 1999) (emphasis supplied). Here, Huston clearly provides no explicit disclosure of a consensus sequence. The only alleged basis for implicit disclosure lies in his reference to framework regions "homologous" to the framework regions of a human immunoglobulin (col. 3, lines 31-32). However, the term "homologous" could mean the frameworks are identical to those of a human immunoglobulin or any minor variation thereof. The term "homologous" does not necessarily mean a consensus sequence, particularly as this term was understood and used by those in the art at the priority date of the claimed invention. In the absence of either explicit disclosure, or an implicit disclosure that meets the requirements for inherency, Huston does not anticipate any of claims 116-132.

Claim 119 and claim 116 (as amended) is distinguished from Huston for the additional reason that the claims require amino acids from the donor replacing corresponding amino acids in the acceptor immunoglobulin framework. Contrary to the Examiner's position, Huston does not disclose such in the context of a humanized immunoglobulin as claimed. This issue was extensively discussed in predecessor case, USSN 08/474,040 (now US 5,693,761). Both of the passages cited by the Examiner as allegedly disclosing replacement of an amino acid in a framework region of an acceptor immunoglobulin from a donor immunoglobulin for the purpose of increasing binding specificity are in fact considerably more general and vague, and do not provide this specific teaching. The two passages are reproduced below:

> Practice of the invention enables the design and biosynthesis of various reagents, all of which are characterized by a region having affinity for a preselected antigenic substance. Other regions of the

> biosynthetic protein are designed with the particular planned utility of the protein in mind. Thus, if the reagent is designed for intravascular use in mammals, the FR regions comprise amino acids similar or identical to at least a portion of the framework region amino acids of antibodies native to that mammalian species. On the other hand, the amino acids comprising the CDRs may be analogous to a portion of the amino acids from the hypervariable region (and certain flanking amino acids) of an antibody having a known affinity and specificity, e.g., a murine or rat monoclonal antibody.
>
> Col. 7, lines 40-54.
>
> By sequencing any antibody, or obtaining the sequence from the literature, in view of this disclosure one skilled in the art can produce a BABS of any desired specificity comprising any desired framework region. Diagrams such as FIG. 3 comparing the amino acid sequence are valuable in suggesting which particular amino acids should be replaced to determine the desired complementarity. Expressed sequences may be tested for binding and empirically refined by exchanging selected amino acids in relatively conserved regions, based on observation of trends in amino acid sequence data and/or computer modeling techniques.
>
> Col. 13, lines 47-57.

Although the first quoted passage does refer to amino acids flanking hypervariable regions, Huston does not use the term hypervariable region synonymously with CDR (see col. 7, lines 20-25). According to Huston, CDRs include flanking amino acids not present in the hypervariable regions (id.). Thus, Huston's reference in the cited passage to amino acids flanking hypervariable regions would be understood as referring to amino acids *within* the CDR region, and not framework amino acids, especially because the relevant sentence of Huston specifically refers to "amino acids comprising the CDRs." The reference in the second quoted passage to Fig. 3 being valuable in suggesting which amino acids should be replaced would likely be understood as referring to replacement of CDR regions. In any event, this passage provides no specific reference to substituting donor residues in the variable region framework. Finally, as the

Examiner acknowledges, the alleged working example (col. 13, lines 16-38) of a variable region framework substitution does not occur within the context of a humanized antibody. For these reasons, Huston does not disclose substituting donor amino acids for corresponding amino acids in the acceptor immunoglobulin framework of a humanized immunoglobulin as specified in claim 119.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400.

Respectfully submitted,

J. Liebeschuetz

Joe Liebeschuetz
Reg. No. 37,505

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 650-326-2400
Fax: 415-576-0300
Attachments
JOL:adm

60248752 v1

Excerpt from paper 32
USSN 08/146,206

#32/g
CAP
10/7/97

Patent Docket P0709P1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Paul J. Carter et al. Serial No.: 08/146,206 Filed: 17 November 1993 For: METHOD FOR MAKING HUMANIZED ANTIBODIES	Group Art Unit: 1816 Examiner: P. Nolan **CERTIFICATE OF HAND DELIVERY** I hereby certify that this correspondence is being delivered to Receptionist, Group 1800 of the United States Patent and Trademark Office, Washington, D.C. 20231 on October ___ 1997 Printed Name:

SUPPLEMENTAL AMENDMENT UNDER 37 C.F.R. §1.111

OCT - 7 1997

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Applicants respectfully request reconsideration of the above-identified application in view of the following amendments and remarks.

IN THE SPECIFICATION:

On page 8, lines 25-27 and page 15, lines 23-24, please replace the sequence in its entirety with the following sequence --
EVQLVESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLEWVAVISENGSDTYYADS
VKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCARDRGGAVSYFDVWGQGTLVTVSS--

On page 9, line 30, please replace "huκI" with --hulII--.

IN THE CLAIMS:

10/10/1997 PSTANBAC 00000021 DAH:070630 08146206
01 FC:103 80.00 CH

10. (Three times amended) A humanized antibody variable domain having a non-human Complementarity Determining Region (CDR) incorporated into a human antibody variable domain, wherein an amino acid residue has been substituted for the human amino acid residue at a site selected from the group consisting of:
4L, [36L], 38L, 43L, 44L, 46L, 58L, 62L, 65L, 66L, 67L, 68L, 69L, [70L,] 73L, 85L, [87L,] 98L, 2H,

4H, [24H,] 36H, [37H,] 39H, 43H, 45H, [49H, 68H,] 69H, 70H, [73H,] 74H, 75H, 76H, 78H and 92H.

Please add the following claims:

--39. A humanized heavy chain variable domain comprising FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein FR1-4 comprise the four framework regions of a consensus human variable domain of a human heavy chain immunoglobulin subgroup and CDR1-3 comprise the three complementarity determining regions (CDRs) of a nonhuman import antibody, and further wherein consensus human framework region (FR) residues have been replaced by nonhuman import residues where the FR residue (a) noncovalently binds antigen directly; (b) interacts with a CDR; (c) comprises a glycosylation site which affects the antigen binding or affinity of the antibody; or (d) participates in the V_L - V_H interface.

40. The humanized heavy chain variable domain of claim 39 wherein the human heavy chain immunoglobulin subgroup is V_H subgroup III.

41. The humanized heavy chain variable domain of claim 40 wherein:
FR1 of the consensus human variable domain comprises the amino acid sequence:
EVQLVESGGGLVQPGGSLRLSCAAS (SEQ ID NO:27);
FR2 of the consensus human variable domain comprises the amino acid sequence:
WVRQAPGKGLEWVA (SEQ ID NO:28);
FR3 of the consensus human variable domain comprises the amino acid sequence:
RFTISRDDSKNTLYLQMNSLRAEDTAVYYCAR (SEQ ID NO:29); and
FR4 of the consensus human variable domain comprises the amino acid sequence:
WGQGTLVTVSS (SEQ ID NO:30).

42. The humanized antibody of claim 22 which lacks immunogenicity upon repeated administration to a human patient in order to treat a chronic disease in that patient.--

REMARKS

A. Amendments

The undersigned confirms having met with Examiners Nolan and Eisenschenk in the interview 7/23/97 and takes this opportunity to thank the Examiners for the courtesies extended in the interview. Claims 39-41 have been added herein which use language as proposed by Examiner Nolan in the interview. Independent claim 39 is similar to a combination of presently pending claims 22 and 23. Basis for the language "FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein FR1-4 comprise the four framework regions of a consensus human variable domain of a human heavy chain immunoglobulin subgroup and CDR1-3 comprise the three complementarity determining regions (CDRs) of a nonhuman import antibody" in claim 39 is found on page 1, lines 28-30 and page 25, lines 28-29, for example. Claim 40 finds specification basis on at least page 15, line 18. Claim 41 finds specification support in Figure 1B with respect to the framework regions of the HUV_HIII consensus sequence therein. Claim 42 has also been added and finds specification basis on at least page 60, lines 25-32 and page 70, lines 6-8. With respect to the amendments to the specification, the sequence on pages 8 and 15 has been corrected (see Section B of this amendment) and the typographical error with respect to the Fig. 5 sequence has been corrected herein. In that the amendments do not introduce new matter, their entry is respectfully requested.

B. Substitute Sequence Listing

A further substitute sequence listing is submitted herewith. Applicants have found that SEQ ID NO:4 in the previous sequence listings did not correspond to the HUV_HIII consensus sequence of Fig. 1B (see page 9, lines 1-2) and hence SEQ ID NO:4 in the attached substitute sequence listing has been corrected accordingly. Furthermore, SEQ ID NO:4 is hereby corrected on pages 8 and 15 of the application. In addition, separate sequence identifiers (SEQ ID NO's 27-30) have been given to the FR1-4 sequences in claim 41 added herein. In accordance with 37 C.F.R. §§1.821(f) and (g), the undersigned hereby states that the content of the paper and the computer readable sequence listings is the same. I further state that this submission includes no new matter.

C. Antibodies humanized according to the teachings of the instant application

As discussed in the interview, the consensus human variable domain of the instant claims has been used to humanize a number of antibodies, including:

1. *Anti-p185^{HER2} antibodies.* See Example 1 of the application, including Table 3 on page 72 (which describes humanized variants huMAb4D5-1-8) and page 65, lines 1-4 (concerning the use of a consensus human variable domain as recited in the claims herein). huMAb4D5-6 and huMAb4D5-8 had binding affinities which were suprisingly *superior* to that of the nonhuman antibody (muMAb4D5); see second to last column of Table 3. Repeated administration of the humanized anti-p185^{HER2} antibody huMAb4D5-8 has not lead to an immunogenic response in cancer patients treated therewith. See abstract of Baselga *et al.*, *J. Clin. Oncol.* 14(3):737-744 (1996), of record.

2. *Anti-CD3 antibodies.* See Example 3 on pages 79-88 of the application; and Fig. 5 as well as page 9, lines 25-31 concerning the use of a consensus human variable domain as claimed herein. [Note: In the Fig. 5 V_H consensus sequence (huIII), the last residue of FR2 is S, *i.e.* A→S, and eighth residue of FR3 is N, *i.e.* D→N, because of changes in 1987 to 1991 consensus sequence of Kabat *et al.*; such an equivalent consensus sequence and other changes in consensus sequences that result from the addition of further human antibody sequences to subsequent antibody compilations by Kabat *et al.* are clearly encompassed by the claims herein]. Humanized anti-CD3 variant (v1) was found to enhance the cytotoxic effects of activated human cytotoxic T lymphocytes (CTL) 4-fold against SK-BR-3 tumor cells overexpressing p185^{HER2} (page 81, lines 1-4). Variants of the humanized v1 antibody were made (v6 to v12; see page 82, line 22 and page 84, line 17 through to page 85, line 2 and page 86, lines 17-31), including the most potent variant, v9, which bound Jurkat cells almost as efficiently as the chimeric BsF(ab')$_2$ (page 86, lines 20-22).

3. *Anti-CD18 antibody.* See Example 4 on page 89 of the application and Figs. 6A and 6B with respect to a consensus human variable domain as claimed in the instant application. The binding affinity of the humanized anti-CD18 antibody (pH52-8.0/pH52-9.0; see Figs. 6A and 6B of

the application) was similar to the nonhuman H52 antibody; *i.e.* the humanized antibody has an affinity of 3.9 ± 0.9nM and murine H52 antibody has an affinity of 1.5 ± 0.3nM.

4. *Anti-IgE antibodies.* See Presta *et al. J. Immunol.* 151(5)2623-2632 (1993), of record. Use of a consensus human variable domain of the claims of the instant application is disclosed on page 2624 (column 1, first and third full paragraphs) and in Fig. 1. A number of humanized variants were made (see full paragraph 2 in column 1 on page 2624), including F(ab)-12 with only five framework region substitutions which exhibited binding comparable to the murine antibody (paragraph 2 on page 2631). Multidose administrations of full length anti-IgE variant 12 did not induce a human antihuman antibody response in allergic patients treated therewith (see column 1, last paragraph on page 311 of Shields *et al.*, *Int. Arch. Allergy Immunol.* 107:308-312 (1995), of record).

5. *Anti-CD11a antibodies.* See Werther *et al. J. Immnol.* 157:4986-4995 (1996), of record. Use of a consensus human variable domain as taught and claimed in the instant application is discussed in the first sentence of the Results section on page 4988 and in Fig. 1 (see note in paragraph 2 above, with respect to changes in 1987 to 1991 consensus sequences. Eight humanized variants were made (see Table 1 on page 4989), including HuIgG1 which had an apparent Kd similar to the parent murine antibody and comparable activity to the murine antibody in the cell adhesion and mixed leukocyte reaction (MLR) assays (see paragraph briging columns 1-2 on page 4993).

6. *Anti-VEGF antibodies.* See Presta *et al.* "Humanization of an anti-VEGF monoclonal antibody for the therapy of solid tumors and other disorders" *Cancer Research*, in press, pps. 1-32 of the manuscript, of record. The first paragraph on page 12 refers to the use of a consensus human variable domain as in the claims of this application. With respect to the consensus sequence in the figure on page 32 of the manuscript, see note in paragraph 2 above concerning change in 1987 to 1991 consensus sequences. As shown in Table 1 on page 29, twelve humanized anti-VEGF antibodies were made. The humanized antibody 12-IgG1 acquired the binding properties and biological activities of a high-affinity murine anti-VEGF MAb (see page 16,

last paragraph of this reference).

D. FR substitutions by Queen *et al.*

With respect to pending claim 10 herein reciting substitutions at specified sites in the V_H and V_L framework regions, as discussed at the interview, Queen *et al. PNAS, USA* 86:10029-10033 (1989) and US Patent 5,530,101 (the "101 patent") (cited by the office in the previous office action) use sequential numbering for the variable domain residues of the antibodies described in these references, whereas the claims of the instant application use Kabat numbering for the framework region residues (see page 14, lines 6-22 of the instant application). As requested by the Examiner in the interview, alignments of heavy chain variable domain (Exhibit A) and light chain variable domain (Exhibit B) sequences of the 101 patent (including the sequences for the murine and humanized anti-Tac antibody of Queen *et al.*) with sequential and Kabat residue numbering are attached. "murx" refers to the murine antibody sequence; "hzx" refers to the humanized antibody sequence; "H" is used for heavy chain variable domain sequences and "L" for light chain variable domain sequences. The sites at which the 101 patent refers to FR substitutions are:

Anti-Tac antibody (Figs. 1A and 1B of 101 patent)			
V_H FR substitions		**V_L FR substitutions**	
Sequential numbering	**Kabat numbering**	**Sequential numbering**	**Kabat numbering**
27H	27H	48L	48L
30H	30H	60L	60L
48H	48H	63L	63L
67H	66H		
68H	67H		
93H	89H		
95H	91H		
98H	94H		

107H	103H		
108H	104H		
109H	105H		
111H	107H		
Fd79 antibody (Figs. 2A and 2B of 101 patent)			
V_H FR substitions		**V_L FR substitutions**	
Sequential numbering	**Kabat numbering**	**Sequential numbering**	**Kabat numbering**
82H	81H	9L	9L
97H	93H	45L	41L
112H	103H	46L	42L
		53L	49L
		81L	77L
		83L	79L
Fd138-80 antibody (Figs. 3A and 3B of 101 patent)			
V_H FR substitions		**V_L FR substitutions**	
Sequential numbering	**Kabat numbering**	**Sequential numbering**	**Kabat numbering**
27H	27H	36L	36L
30H	30H	48L	48L
37H	37H	63L	63L
48H	48H	87L	87L
67H	66H		
68H	67H		
93H	89H		
98H	94H		

111H	103H		
112H	104H		
113H	105H		
115H	107H		
M195 antibody (Figs. 4A and 4B of the 101 patent)			
V_H FR substitions		**V_L FR substitutions**	
Sequential numbering	**Kabat numbering**	**Sequential numbering**	**Kabat numbering**
27H	27H	10L	10L
30H	30H	40L	36L
48H	48H	52L	48L
67H	66H	67L	63L
68H	67H	74L	70L
93H	89H	110L	106L
95H	91H		
98H	94H		
106H	103H		
107H	104H		
108H	105H		
110H	107H		
mik-β1 antibody (Figs. 5A and 5B of the 101 patent)			
V_H FR substitions		**V_L FR substitutions**	
Sequential numbering	**Kabat numbering**	**Sequential numbering**	**Kabat numbering**
1H	1H	13L	13L
29H	29H	41L	42L

30H	30H	70L	71L
49H	49H		
72H	72H		
73H	73H		
84H	82bH		
89H	86H		
90H	87H		
CMV5 antibody (Figs. 6A and 6B of the 101 patent)			
V_H FR substitions		**V_L FR substitutions**	
Sequential numbering	**Kabat numbering**	**Sequential numbering**	**Kabat numbering**
5H	5H	49L	49L
24H	24H		
27H	27H		
28H	28H		
30H	30H		
69H	68H		
80H	79H		
97H	93H		
AF2 antibody (Figs. 44A and 44B of the 101 patent)			
V_H FR substitions		**V_L FR substitutions**	
Sequential numbering	**Kabat numbering**	**Sequential numbering**	**Kabat numbering**
27H	27H	48L	48L
28H	28H	63L	63L
30H	30H	70L	70L

93H	89H		
95H	91H		
98H	94H		
107H	103H		
108H	104H		
109H	105H		
111H	107H		

Should the Examiner have any comments or questions concerning this amendment, he is invited to call Wendy Lee at (650) 225-1994 concerning these.

Respectfully submitted,
GENENTECH, INC.

Date: October 6, 1997

By: WMLee
Wendy M. Lee
Reg. No. 40,378

1 DNA Way
So. San Francisco, CA 94080-4990
Phone: (650) 225-1994
Fax: (650) 952-9881